

Application No.: 10/084,706  
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**Listing of Claims:**

The following listing of claims replaces all prior versions and listings of claims in the application:

1.-87. (Canceled)

88. (New) An interferon  $\beta$  polypeptide variant exhibiting interferon  $\beta$  activity, comprising a variant sequence which differs from the wild-type human interferon  $\beta$  sequence SEQ ID NO:2 in no more than 15 amino acid residues, the variant sequence comprising (a) at least one N-glycosylation site and (b) an amino acid substitution at position -1 relative to at least one of the N-glycosylation site(s).

89. (New) The variant according to claim 88, wherein at least one of the N-glycosylation site(s) is a naturally occurring N-glycosylation site.

90. (New) The variant according to claim 88, wherein at least one of the N-glycosylation site(s) is an introduced N-glycosylation site.

91. (New) The variant according to claim 90, wherein the introduced N-glycosylation site is in a position that in wild-type human interferon  $\beta$  is occupied by an amino acid residue having at least 25% of its side chain exposed to the solvent.

92. (New) The variant according to claim 90, wherein the variant comprises at least two introduced N-glycosylation sites

93. (New) The variant according to claim 92, comprising an amino acid substitution at position -1 relative to at least one of the introduced N-glycosylation site(s).

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94. (New) The variant according to claim 88, further comprising at least one non-polypeptide moiety covalently attached to the variant.

95. (New) The variant according to claim 94, comprising at least one sugar moiety and at least one polymer molecule.

96. (New) The variant according to claim 95, wherein at least one of the polymer molecule(s) is covalently attached to a lysine residue of the variant.

97. (New) The variant according to claim 95, wherein at least one of the polymer molecule(s) is covalently attached to the N-terminus of the variant.

98. (New) The variant according to claim 95, wherein the polymer molecule comprises a linear polyethylene glycol or a branched polyethylene glycol.

99. (New) A composition comprising the variant of claim 88 or 94 and a pharmaceutically acceptable diluent, carrier, or excipient.

100. (New) A nucleic acid comprising a nucleotide sequence encoding the variant of claim 88.

101. (New) An expression vector comprising the nucleic acid of claim 100.

102. (New) A glycosylating host cell comprising the expression vector of claim 101.

103. (New) The glycosylating host cell according to claim 102, selected from the group consisting of an *S. cerevisiae* cell, a *Yarrowia pastoris* cell, a CHO cell, a BHK cell, an FHK cell, and an SF9 cell.

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104. (New) A method of making a variant, the method comprising: providing a culture comprising a glycosylating host cell, the glycosylating host cell comprising a nucleotide sequence which encodes the variant of claim 88, culturing the culture under conditions which permit expression and glycosylation of the variant, and recovering the variant.

105. (New) The method according to claim 104, wherein the glycosylating host cell is selected from the group consisting of an *S. cerevisiae* cell, a *Pichia pastoris* cell, a CHO cell, a BHK cell, an HEK cell and an SF9 cell.

106. (New) The method according to claim 104, further comprising attaching at least one non-polypeptide moiety to the variant, wherein the non-polypeptide moiety comprises a polymer molecule.

107. (New) The method according to claim 106, wherein the polymer molecule comprises a linear polyethylene glycol or a branched polyethylene glycol.

108. (New) A method for producing a variant IFNB molecule exhibiting increased *in vivo* N-glycosylation relative to a parent IFNB molecule, the parent IFNB molecule comprising a parent IFNB sequence comprising at least one N-glycosylation site, which method comprises:

- i) introducing a mutation into a parent polynucleotide that encodes the parent IFNB sequence, to produce a variant polynucleotide that encodes a variant IFNB sequence, the mutation introducing into the variant IFNB sequence an amino acid substitution at position -1 relative to at least one of the N-glycosylation site(s) of the parent IFNB sequence,
- ii) expressing the variant polynucleotide to produce the variant IFNB molecule, and the parent IFNB polynucleotide to produce the parent IFNB molecule, in glycosylating host cell cultures under comparable conditions,
- iii) comparing the degree of glycosylation of the variant IFNB molecule and the parent IFNB molecule, and

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- iv) if necessary, repeating steps i) and ii), each time substituting a different amino acid at position -1 into the variant IFNB sequence, until a variant IFNB molecule exhibiting increased *in vivo* N-glycosylation relative to the parent IFNB molecule is obtained.